Environmental Health Perspectives
Vol. 81, pp. 77-79, 1989

Mutagenic Metabolites of Benzene Detected in the Microscreen Assay

by Toby G. Rossman,* Catherine B. Klein,* and Carroll A. Snyder*

The reactive metobolite responsible for benzene hematotoxicity and carcinogenicity is unknown. It can be hypothesized that the ultimate carcinogen derived from benzene metabolism might also act as a mutagen. This laboratory has recently developed a new assay that can detect mutagens of all types, using a single strain of bacteria, *E. coli* WP2s (λ), as a target. Different genetic end points can be monitored in the same exposed population of bacteria. When a number of known metabolites of benzene were assayed, only *trans,trans*-muconic acid gave a strong positive response. Mutations were induced at two genetic loci (Trp^+ revertants and T5 resistance). The mutagenic activity was greatly increased when a rat liver metabolizing system was added. We speculate that *trans,trans*-muconic acid is metabolized to a diepoxide, which may be the ultimate mutagen and possibly the ultimate carcinogen.

Introduction

Human exposure to benzene is associated with leukemias, particularly acute myeloblastic leukemia and its variants (*1*). In animal studies, leukemias and Zymbal's gland tumors are observed in rats and mice after exposure to benzene. Other tumors seen include those of skin and oral cavity in rats, and lung, preputial gland, mammary gland, and malignant lymphoma in mice (*2*). Benzene-induced chromosomal abnormalities have been produced in mice, with male mice showing more susceptibility than female mice (*6-8*). Sister chromatid exchanges are also produced by exposure to benzene (*7*).

Workers exposed to low levels of benzene (less than 10 ppm) were found to have increased chromosomal abnormalities in their peripheral lymphocytes (*3-5*).

There is substantial evidence showing that, although benzene itself cannot react with DNA or cause mutations, benzene can be metabolized to genotoxic agents. When radiolabeled benzene is administered by inhalation, DNA adducts are found in the liver (*9*). Incubation of bone marrow mitochondria with labeled benzene resulted in seven guanine adducts and two adenine adducts (*10*). Benzene is not mutagenic in the Ames test (*11*), even with metabolic activation, suggesting that the rat liver activation system is unable to metabolize benzene to a mutagenic compound. However, bone marrow enzymes can apparently metabolize benzene to products that can form DNA adducts (*10*).

Results and Discussion

This laboratory has recently developed a new short-term *in vitro* assay to detect mutagens of all classes using only one strain of bacteria (*12-14*). The main features of this assay are shown in Figure 1. Serial dilutions of the test compound are added to microtiter wells that are then inoculated with *E. coli* WP2s (λ). This strain carries a *uvrA* mutation, which renders the bacteria unable to carry out excision repair of bulky adducts; a mutation in the *trpE* gene, which makes the strain dependent upon exogenous tryptophan for growth. After overnight growth in the presence of the test agent, aliquots are taken from the subtoxic wells and assayed for a number of genetic end points.

Some of the metabolites of benzene are shown in Figure 2. When these and some related compounds were assayed in the Microscreen, only one compound, *trans,trans*-muconic acid (ttMA), was very active. Results with the T5-resistance marker are shown in Figure 3. It is clear that although slight mutagenic activity can be detected with ttMA alone, a great enhancement is seen when rat liver S9 is added. Results with the direct-acting agent β-propiolactone (BPL) are shown for comparison.

*Institute of Environmental Medicine, New York University Medical Center, 550 First Avenue, New York, NY 10016.

Address reprint requests to T. G. Rossman, Institute of Environmental Medicine, New York University Medical Center, 550 First Avenue, New York, NY 10016.

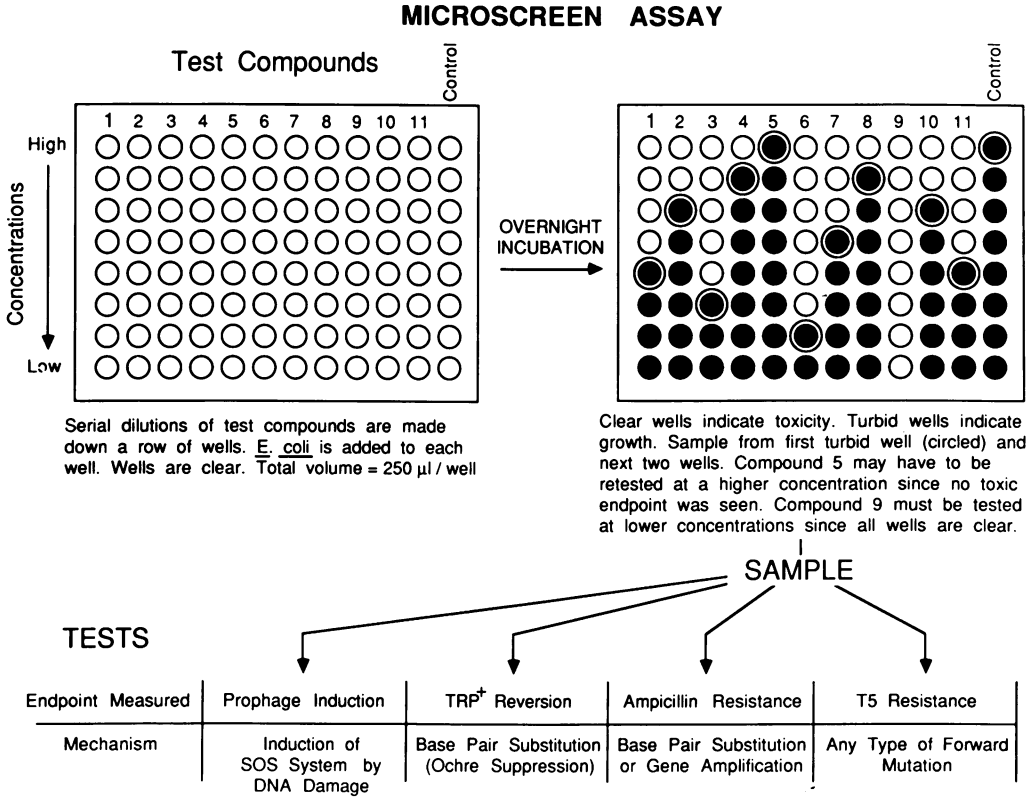

FIGURE 1. Microscreen assay.

BPL appears about 10-fold more active than ttMA + S9. However, since the molecular weight of ttMA is twice that of BPL, on a molar basis BPL is about five times more active than metabolized ttMA.

Table 1 summarizes the results from the three mutagenesis end points in the Microscreen. The slight activity with ttMA alone is detected at the same genetic loci as the much greater activity seen in the presence of rat liver S9, suggesting that the *E. coli* might be able to metabolize ttMA to the same product(s) to a lesser extent. The ability to cause Trp^+ reversion indicates that a base pair substitution mutagen is formed. Acrylic acid, which contains a single α, β unsaturated acid moiety, is not active. It is of interest that benzene itself caused a slight increase in ampicillin-resistant mutants. This activity was not enhanced by rat liver S9 (Table 1). Since this

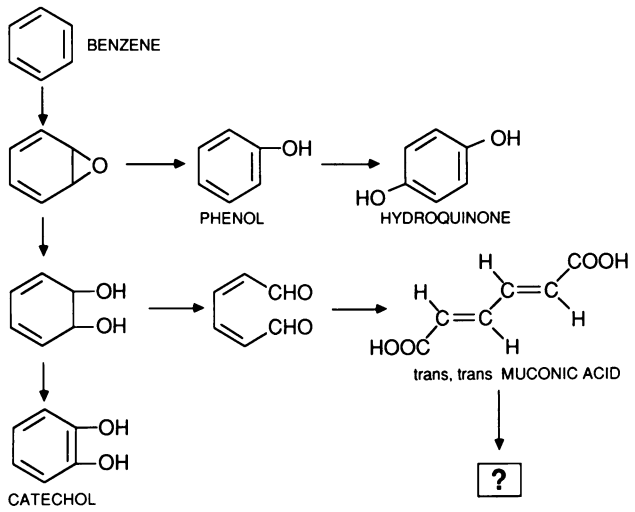

FIGURE 2. Some of the benzene metabolites tested for mutagenicity.

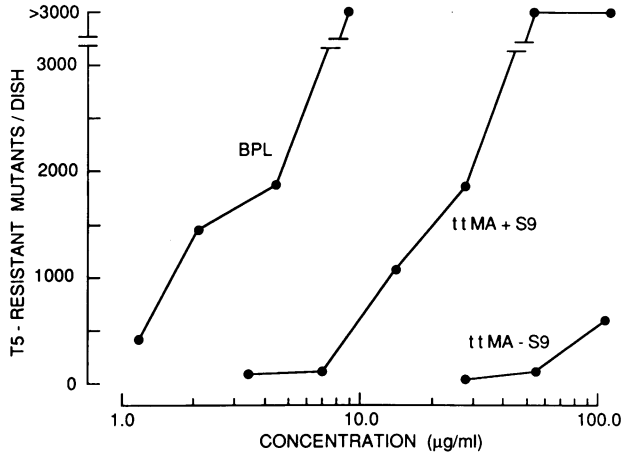

FIGURE 3. Mutagenicity (to T5 resistance) of *trans,trans*-muconic acid (ttMA) with and without metabolic activation by Aroclor-induced rat liver S9. Results with β-propiolactone (BPL) are included for comparison.

Table 1. Toxicity and mutagenicity of benzene metabolites and related compounds.

Compound	S9 activation	Toxicity, μg/mL	Mutagenicity		
			Trp$^+$	Amp^r	T5^r
Benzene	–	50	–	+	–
	+	50	–	+	–
Hydroquinone	–	62	–	–	–
	+	500	–	–	–
Catechol	–	500	–	–	–
	+	500	–	–	–
Resorcinol	–	2500	–	–	–
	+	2500	–	–	–
trans,trans-Muconic acid	–	(NT 110)[a]	+	–	+
	+	(NT 110)[a]	++++	–	++++
Acrylic acid	–	96	–	ND[b]	–
	+	96	–	ND	–

[a]Not toxic at 110 μg/mL. Higher concentrations were insoluble.
[b]ND, not determined.

marker can detect gene amplification (*15*), it is possible that benzene itself can induce gene amplification by a mechanism that does not involve DNA adducts.

We speculate that the ultimate mutagenic agent(s) derived from benzene is one or more diepoxides formed from ttMA. These compounds would be analogues of diepoxybutane, but with two carboxyl groups. Diepoxybutane is a known animal carcinogen (*16*).

Our data suggest that the liver is unable to form ttMA from benzene, but if ttMA is formed elsewhere, liver enzymes are able to metabolize it further to mutagenic metabolites. It is possible that bone marrow enzymes are able to produce ttMA and its metabolites from benzene. If so, this would explain the organ specificity of benzene toxicity and carcinogenicity.

We acknowledge the fine technical assistance of Jennifer Bergoine and thank Eleanor Cordisco for help in preparing this manuscript. This work was supported by grant 811536 from the U.S. Environmental Protection Agency, by grant 15-111 from the March of Dimes, and in part by Center grant ES-00260 from the National Institute of Environmental Health Sciences, Center grant CA-13343 from the National Cancer Institute and, Special Institutional grant 00009 from the American Cancer Society.

REFERENCES

1. Goldstein, B. D. Hematotoxicity in humans. In: Benzene Toxicity, A Critical Evaluation (S. Laskin and B. Goldstein, Eds.), J. Toxicol. Environ. Health, Suppl. 2: 69-105 (1977).
2. Snyder, C. A. Benzene. In: Ethel Browning's Toxicity and Metabolism of Industrial Solvents, Vol. I., Hydrocarbons (R. Snyder, Ed.), Elsevier Science Publishers, New York, 1987, pp. 3-37.
3. Picciano, D. Cytogenetic study of workers exposed to benzene. Environ. Res. 19: 33-38 (1979).
4. Sarts, F., Cominato, I., Pinton, A., Brovedami, P., Merler, E., Peruzzi, M., Bianchi, V., and Lewis, A. A cytogenetic study on workers exposed to low concentrations of benzene. Carcinogenesis 5: 827-832 (1984).
5. Watanabe, T., Endo, A., Kato, Y., Shima, S., Watenabe, T., and Ikeda, M. Cytogenetics and cytokinetics of cultured lymphocytes from benzene-exposed workers. Int. Arch. Occup. Environ. Health 46: 31-41 (1980).
6. Meyne, J., and Legator, M. Sex-related differences in cytogenetic effects of benzene in the bone marrow of Swiss mice. Environ. Mutagen. 2: 43-50 (1980).
7. Tice, R., Costa, D., and Drew, R. Cytogenetic effects of inhaled benzene in murine bone marrow: Induction of sister chromatid exchanges, chromosome aberrations, and cellular proliferation inhibition in DBA/2 mice. Proc. Natl. Acad. Sci. (U.S.) 77: 2148-2152 (1980).
8. Siou, G., Conan, L., and El Haitem, M. Evaluation of the clastogenic action of benzene by oral administration with two cytogenetic techniques in mouse and Chinese hamster. Mutat. Res. 90: 273-278 (1981).
9. Lutz, W., and Schlatter, C. Mechanism of the carcinogenic action of benzene: Irreversible binding to rat liver DNA. Chem.-Biol. Interact. 18: 241-245 (1977).
10. Rushmore, T., Snyder, R., and Kalf, G. Covalent binding of benzene and its metabolites to DNA in rabbit bone marrow mitochondria *in vitro*. Chem.-Biol. Interact. 49: 133-154 (1984).
11. DeFlora, S., Zarracchi, P., Camoirano, A., Bennicelli, C., and Badveati, G. S. Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutat. Res. 133: 161-198 (1984).
12. Rossman, T. G., Meyer, L. W., and Molina, M. The genetic toxicology of metal compounds. I. Induction of λ prophage in *E. coli* WP2s (λ). Environ. Mutag. 6: 59-69 (1984).
13. Rossman, T. G., Meyer, L. W., Butler, J. P., and Daisey, J. M. Use of the Microscreen assay for airborne particulate organic matter. In: Short-Term Bioassays in the Analysis of Complex Environmental Mixtures IV (M. D. Waters, S. S. Sandhu, J. Lewtas, L. Claxton, G. Strauss, and S. Nesnow, Eds.), Plenum Press, New York, 1985, pp. 9-23.
14. Rossman, T. G., Meyer, L. W., and Molina, M. Induction of λ prophage as a screen for genotoxic agents. Ann. N. Y. Acad. Sci. 463: 347-348 (1986).
15. Edlund, T., Grundstrom, T., and Normark, S., Isolation and characterization of DNA repetitions carrying the chromosomal β-lactamase gene of *Escherichia coli* K-12. Mol. Gen. Genet. 173: 115-125 (1979).
16. IARC Monographs. On the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 11. International Agency for Research on Cancer, Lyon, France, 1976.